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ICAM-1 expression and leukocyte accumulation in inner stripe of outer medulla in early phase of ischemic compared to $HgCl_2$-induced ARF

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ICAM-1 expression and leukocyte accumulation in inner stripe of outer medulla in early phase of ischemic compared to $HgCl_2$-induced ARF.

Background. After ischemia/reperfusion (I/R), as well as after toxic insults, there is significant infiltration of leukocytes in the kidney. It is well known that antibodies against adhesion molecules [e.g., intercellular adhesion molecule-1 (ICAM-1)] protect the kidney against acute ischemic injury. In contrast, same antibody treatment did not protect the rat kidney against toxic acute renal failure (ARF) induced by $HgCl_2$. Protection obtained by anti-adhesion treatment in I/R injury is an early phenomenon, since delaying the administration of anti-ICAM-1 for 8 hours did not protect the kidney anymore. The aim of this study was to compare the early ICAM-1 expression and leukocyte accumulation in different zones of ischemic and toxic injury.

Methods. Male Lewis rats were injected with $HgCl_2$ (2 mg/kg, subcutaneously) or uninephrectomized Lewis rats were submitted to 30°C warm ischemia (I/R injury). Rats were sacrificed at 2, 6, 12 and 24 hours. ICAM-1 (1A29) expression in kidney was evaluated morphometrically. Different subsets of leukocytes were stained by immunohistochemistry and counted in cortex, the outer stripe of the outer medulla (OSOM) and the level of the inner stripe of the outer medulla (ISOM).

Results. Although the functional and morphologic damage was comparable between the I/R and toxic ARF group, different ICAM-1 expression could be observed early after injury. ICAM-1 expression in the ISOM started already 2 hours after the onset of I/R injury, and was increased after 12 hours in the cortex and after 24 hours in the OSOM.

In contrast, during the first 24 hours after injury, ICAM-1 expression in $HgCl_2$-injured kidneys was not different from noninjured kidneys in the ISOM and the cortex, whereas in the OSOM, ICAM-1 expression increased. The number of polymononuclear cells (PMNs) was low in noninjured kidneys and did not increase in time after both I/R injury and after $HgCl_2$-induced ARF. In the ISOM, significant monocyte and T-cell accumulation was observed early after I/R but not after $HgCl_2$. There was no significant T-cell accumulation in the cortex or in the OSOM.

Conclusion. After $HgCl_2$, almost no leukocyte accumulation and up-regulation of ICAM-1 was observed the first 12 hours after injury. In contrast, very early after I/R injury, increased expression of ICAM-1 goes along with monocyte and T-cell accumulation in the ISOM, endorsing this particular zone as critical in renal I/R injury. These observations contribute to the understanding why anti-ICAM-1 treatment in acute I/R injury is successful, but fails in acute toxic injury induced by $HgCl_2$.

Key words: ICAM-1, ischemia/reperfusion, leukocytes.

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A prominent infiltration of leukocytes at the site of injury appears to be a common feature, following acute renal injury [1–3]. Until recently, this leukocyte infiltrate, particularly the polymononuclear (PMN) cells among them, was considered as a cause of damage, exacerbating renal injury in the reperfusion phase. Infiltrating leukocytes as scavengers of apoptotic cells or necrotic cellular debris, and as possible source of growth factors [4, 5] suggest a role in the repair process after acute renal failure (ARF). Taken these results, the role of these inflammatory leukocytes in toxic as well as in ischemic ARF remains still controversial.

Recent breakthroughs in leukocyte adhesion molecule research have prompted the re-evaluation of the role of leukocytes in the pathophysiology of ischemic ARF [6]. From the growing series of studies on the role of leukocyte adhesion molecules, particularly intracellular adhesion molecule–1 (ICAM-1) [7–11], have been shown to mediate renal ischemic/reperfusion (I/R) injury. The protection obtained by this antiadhesion approach [anti-ICAM-1/anti-leukocyte function-associated molecule (LFA-1)] in I/R injury is an early phenomenon. Delaying the anti-ICAM-1 administration for 2 hours after I/R injury still could protect the kidney. Delaying, however, the administration of anti-ICAM-1 for 8 hours did not protect the kidney anymore [7–11]. Recently, the same antibody treatment against adhesion molecules was investigated in

toxic-mediated injury [12]. Kelly et al found a protective effect after cisplatin-induced ARF [13], whereas the same anti-adhesion approach used in a $HgCl_2$-induced model of ARF failed to protect the kidney [12].

At first glance, these different results are surprising [1, 2]. After $HgCl_2$, a transient ARF develops, accompanied with necrotic lesions observed in the S_3 segment of the proximal tubule within 24 hours [14]. Similar lesions at the same nephron segment were found after I/R injury [1, 15]. In both models, a leukocyte infiltrate is already visible one day after injury, and becomes most prominent after 5 to 6 days. This leukocyte infiltrate consists in monocytes/macrophages (day 4 and day 5) and helper T cells (day 6 and day 7) and is localized mainly in the zone of damage [1, 2]. The number of PMNs was low and remained so [1, 12].

This paper describes important differences in ICAM-1 up-regulation and adhesion/infiltration of leukocyte subsets in particular zones of the kidney after ischemic and toxic ($HgCl_2$) injury, explaining the difference of the antiadhesion treatment in both models.

METHODS

Animals

After overnight fasting, all procedures were carried out under anesthesia with sodium penthobarbitone (60 mg/kg) in inbred male LEW rats (220 to 260 g). Animals were randomly assigned to three groups: (*1*) control animals; (*2*) animals with acute I/R injury; and (*3*) animals with acute toxic injury, induced by $HgCl_2$.

Control animals. The controls in the present study were normal rat kidneys without injury and received sham surgery. Another group of animals received sham injections. The diluent for mercury is phosphate-buffered saline (PBS). In both groups, no effect of sham surgery or sham injection was observed at any time point. Since results are presented clearly, both groups were assigned as "controls."

Animals with acute I/R injury. A midline abdominal incision was made. Body temperature was kept constantly during ischemia. Careful dissection was carried out to preserve the blood supply to the adrenal glands. Ischemia was performed by cross-clamping the left renal pedicle for 30 minutes with a microvascular clamp, followed by right nephrectomy at the end of the ischemia period. The kidneys were inspected for ischemia as well for good reperfusion for 2 minutes. Postoperative animals were allowed to recover, each in a separate cage, at constant temperature (18°C) and humidity (45%) on a 12-hour light/dark cycle. They received free tap water ad libitum and standard laboratory rat pellets.

Acute toxic injury. At time 0, rats received single subcutaneous injection of 2 mg/kg mercuric chloride. Sacrification of four animals per experimental time point was done at hour 2, 6, 12, and at 24 hours after the onset of injury. Blood samples were taken by heart puncture. All procedures were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals No. 85-23 (1985), and with approval of the Ethical Committee of the University of Antwerp.

Biochemical determinations

Blood samples were allowed to clot and were centrifuged at high speed for 15 minutes. Serum was obtained and stored at −20°C until use. Serum creatinine values were determined in duplicate using a colorimetric method as modified by Jaffé (Creatinine Merckotest, Diagnostica Merck, Germany).

Tissue collection and fixation

Immediately after sacrifice, tissue from the left kidney was collected for different analyses. After dissection of the capsular fat, the kidney was weighed. Five sagittal tissue sections (1 mm thick) were made and fixed in formalin calcium, methacarn and Dubosq Brazil fixative. Two sections were stored in liquid nitrogen.

Morphologic analysis of tubular injury, regeneration, and proliferation

The degree of injury in different tubular compartments was established on periodic acid-Schiff (PAS) reagent-hematoxylin-proliferating cell nuclear antigen (PCNA)-stained sections of methacarn-fixed and paraffin-embedded renal tissue. Proliferation was determined by immunohistochemical staining for the PCNA using the PC10 monoclonal antibody (DAKO, Glostrup, Denmark). Sections were counterstained with PAS. Nuclei were stained with methyl green. Histologic damage of the kidney was scored semiquantitatively. Fifty tubules in the outer stripe of the outer medulla (OSOM) (most sensitive zone for ischemic injury) were assigned using a score-system ranging from 0 to 4 [score 0, normal tubule; score 1, (limited to) loss of brush border; score 2, <50% tubular damage meaning less than 50% of naked basal membrane; score 3, >50% tubular damage; and score 4, total destruction of all epithelial cells, naked basement membrane].

Staining procedure

Immunohistochemical stainings on control rat spleen sections and kidney sections were performed by a panel of monoclonal antibodies, reactive with different subpopulations of leukocytes as described above. The staining with the monoclonal HIS17 (T cells) and ED-1 (monocytes/macrophages) was performed on methacarn-fixed paraffin sections. Staining with the monoclonal antibody OX-8 (CD8+ T cells) and 1A29 (ICAM-1) was performed on formalin-calcium-fixed frozen sections.

Paraffin-embedded slides. Sections (4 μm), prefixed in methacarn for 4 hours, were mounted on poly-L-lysine–

coated microscopic slides. After washing in Tris saline buffer (TSB), 0.01 mol/L Tris-HCl pH 7.6 in 0.9% NaCl) and treatment with normal horse serum (1/5), the sections were incubated overnight with the primary antibodies in different dilutions (HIS17, 1/1000, ED-1, 1/15000).

Cryosections. CD8+ lymphocytes and ICAM-1 was demonstrated on 5 μm renal tissue cryosections prefixed for 90° in 4% formaldehyde (BDH Chemical Ltd., Poole, UK) buffered with 0.1 mol/L Na-cacodylate pH 7.4 containing 1% $CaCl_2$. Sections were treated for 5 minutes with 0.003% trypsin III (Sigma Chemical Co., St. Louis, MO, USA) in 10 mmol/L Tris-HCl buffer pH 7.3 and incubated overnight with the primary antibodies in different dilutions (OX-8, 1/400; 1A29, 1/300). These appropriate antibody dilutions were determined in preliminary experiments. Endogenous tissue peroxidase activity was inhibited by immersion in methanol for 15 minutes, followed by 30 minutes incubation with 0.03% H_2O_2 in TSB.

All sections were incubated with a horse antimouse secondary antibody, biotinylated for 30 minutes, and washed with TSB. The sections were incubated for 60 minutes with the avidin-biotin complex (Vectastain, Vector Laboratories, Burlingame, CA, USA). Washing (3 × TSB) preceded the preparation for color development. The peroxidase substrate solution [20 mg 3-amino-ethylcarbazole (Sigma) in 24 mL dimethyl sulfoxide, 200 mL acetate, pH 5 to 5.2 and 4 mL 0.3% H_2O_2] was added. The sections were lightly counterstained with methyl green and covered in glycerin-gelatin. Positively stained cells were quantified in 22 randomly chosen microscopic fields (magnification ×250) in cortex, OSOM, and inner stripe of the outer medulla (ISOM), expressed as positive cells per mm^2.

Identification/quantification methods of PMNs

Histochemical detection of PMN cells [1] was performed on methacarn-fixed, paraffin-embedded renal tissue sections. The hematoxylin-eosin staining was used to identify and to quantify the infiltration of PMN cells, based upon the localization of the cell and morphology of the nucleus of the cell. Positively stained cells were quantified in 75 randomly chosen microscopic fields (magnification ×400) in cortex, OSOM, and ISOM, expressed as positive cells per mm^2.

ICAM-1 expression in the injured kidney

The expression of ICAM-1, using the monoclonal antibody 1A29 [16], was quantified with an image analysis system (Leitz-Aristoplan microscope, WildLeitz Co., Rockleigh, NJ, USA) with a KS-420 color image analysis system (Kontron, Nashville, TN, USA). ICAM-1 expression in kidney sections at the level of the cortex, the OSOM and the level of the ISOM was measured. Under ×250 magnification, a picture was captured by a video camera and transferred digitally into the computer. Then, ICAM-1 was identified by adjustment of a multicolored threshold in the analysis program. This threshold was set once and readjustment for next slides was not performed. All slides were measured during one session with the same microscope adjustments.

ICAM-1 expression is expressed as percent of positive pixels and as number of positive fields, determined as a unit of connected positive pixels. Area% is a feature of total amount of positive pixels, but gives no information on the pattern of the positive pixels. Number of fields provides information on the coherention of the positive pixels. Ten measurements in different areas in each slide were performed and mean values of readings were obtained.

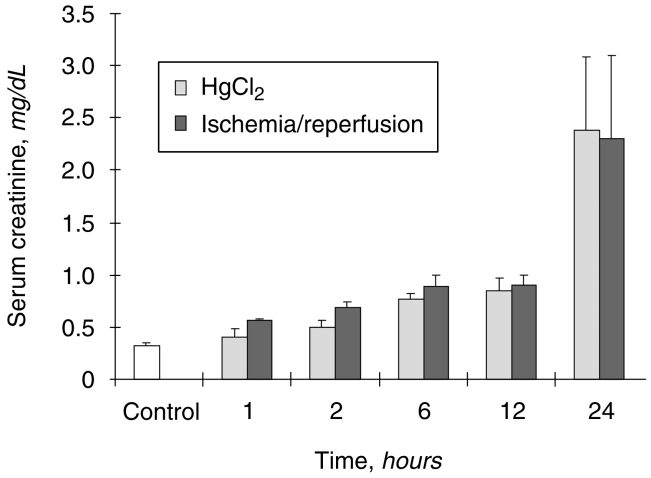

Fig. 1. Serum creatinine. The time course as well as the extent of functional deterioration after injury (serum creatinine at hour 24, $HgCl_2$ = 2.4 ± 0.3 mg/dL; ischemia/reperfusion [I/R] injury = 2.3 ± 0.8 mg/dL, NS) was comparable in both groups.

Statistical analysis

Data are presented as means ± SEM. They were compared with an one-way analysis of variance (ANOVA) analysis and a Student-Newman-Keuls test was used to prove qualitative differences by using the software package SPSS. Significant differences were anticipated when $P < 0.05$.

RESULTS

Renal function

The time course as well as the extent of functional deterioration after injury [serum creatinine at hour 24 ($HgCl_2$ = 2.4 ± 0.3 mg/dL; I/R injury = 2.3 ± 0.8 mg/dL, NS)] as comparable in both groups (Fig. 1).

Renal morphology

The evolution of kidney damage was scored semiquantitatively (Figs. 2 and 3). No signs of damage were ob-

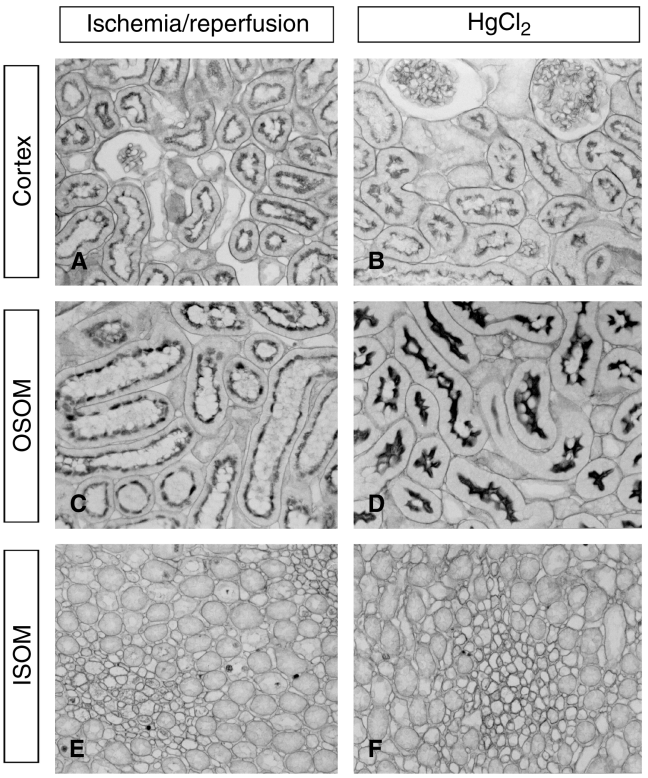

Fig. 2. Kidney morphology, 6 hours after injury [left, Ischemia/reperfusion (I/R) injury group; right, $HgCl_2$ group]. (*A* and *B*) Cortex. (*C* and *D*) The outer stripe of the outer medulla (OSOM). (*E* and *F*) The inner stripe of the outer medulla (ISOM). Renal morphology was comparable in both groups. After I/R injury, a slight decrease in brush border height can be noticed in the proximal tubular cells in the OSOM. Six hours after injury, no signs of tubular necrosis/obstruction were observed in both models of acute renal failure (ARF).

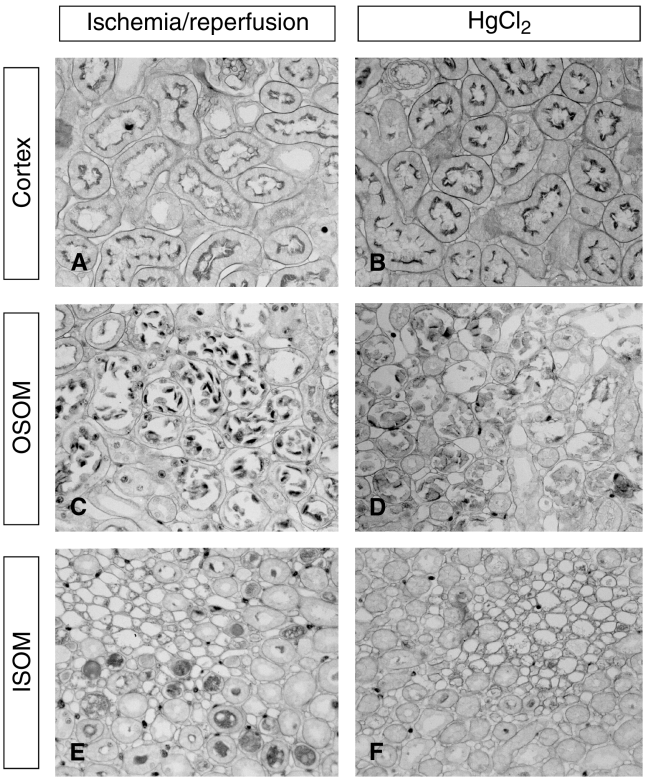

Fig. 3. Kidney morphology, 24 hours after injury. Renal morphology was comparable in both groups. In the cortex, no tubular cell necosis is present. In both models of acute renal failure (ARF), signs of tubular necrosis can be noticed at the level of the proximal tubular cells of the S_3 segment in the outer stripe of the outer medulla (OSOM). In the inner stripe of the outer medulla (ISOM), casts can be found in the lumen of the tubules (left, ischemia/reperfusion (I/R) injury group; right, $HgCl_2$ group. (*A* and *B*) Cortex. (*C* and *D*) OSOM. (*E* and *F*) ISOM.

served in the normal kidney. After both models of ARF, the S_1 and the S_2 segment of the proximal tubular cells remained intact during the first 24 hours after the insult. The first lesions in the S_3 segment of the proximal tubular cells were seen at 12 hours after I/S injury in the OSOM and medullary rays. Some of the brush borders of this segment were shed into the lumen of the tubules. The first lesions in the $HgCl_2$ model were observed 24 hours after the injection. At that time, almost half of the tubules showed a complete detachment of their epithelial cells from their basement membrane. In both models, the extent of tubular necrosis in the S_3 segment was not different. No signs of regeneration were observed within the first 24 hours.

One day after both types of injury, some proteinaceous material was retained in the thin limbs of the tubules. However, in the first 12 hours after injury, there was no evidence of tubular obstruction in the ISOM. No frank necrosis and no tubular casts were found in this region in both ARF groups.

Expression of ICAM-1

The cortex. ICAM-1 expression is demonstrated in Figures 4 and 5. Basal expression in normal kidneys, as well as after injury in both models, was due to the localization of ICAM-1 on some brush borders of proximal tubular cells (noninjured kidneys: number of fields = 156 ± 38, area% = 1.66 ± 0.7%). In the glomerulus, a weak staining could be observed. In cortex, ICAM-1 was significant increased at hour 12 and hour 24 in the I/R group compared to $HgCl_2$. Number of fields and area% of ICAM-1^+ fields were significantly increased in the I/R model 12 hours after reperfusion (number of fields of ICAM-1 at hour 12, $HgCl_2$ = 131 ± 10; I/R injury = 306 ± 7; $P < 0.0005$) (area% of ICAM-1 at hour 12, $HgCl_2$ = 0.7 ± 0.2%; I/R injury = 3.6 ± 0.6%; $P < 0.05$) (Figs. 4 and 5). Also, after 24 hours of reperfusion, the number of fields and area% of ICAM-1^+ fields were significantly increased (number of fields of ICAM-1 at hour 24, $HgCl_2$ = 191 ± 22; I/R injury = 482 ± 74; $P < 0.05$) (area% of ICAM-1 at hour 24, $HgCl_2$ = 1 ± 0.2%; I/R injury = 8 ± 2%; $P < 0.05$). This increased ICAM-1

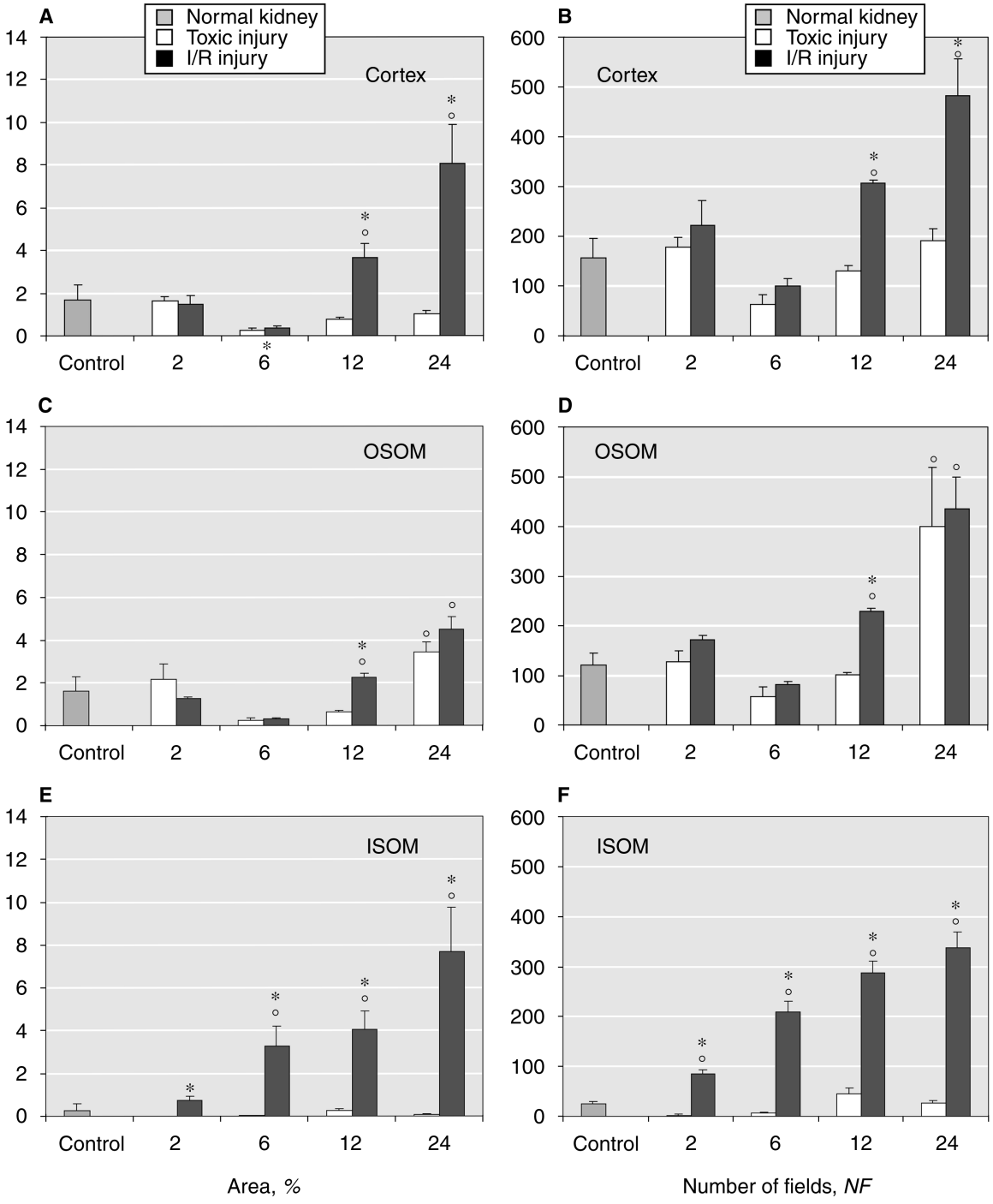

Fig. 4. Expression of ICAM-1 in the different compartments of the kidney after toxic and ischemic acute renal injury. In the cortex (*A* and *B*), intercellular adhesion molecule-1 (ICAM-1) expression is increased 12 hours after ischemic injury. ICAM-1 expression after $HgCl_2$-induced acute renal failure (ARF) is not increased. In the outer stripe of the outer medulla (OSOM) (*C* and *D*), no differences between both groups were observed at 24 hours. In both models, ICAM-1 in the OSOM increased 24 hours after injury, compared to normal kidneys. In the inner stripe of the outer medulla (ISOM) (*E* and *F*), ICAM-1 is up-regulated soon after ischemia/reperfusion (I/R) injury. No increase in ICAM-1 expression could be found after $HgCl_2$-induced ARF. $^*P < 0.05$, I/R injury kidneys vs. $HgCl_2$; $^\circ P < 0.05$, injured kidneys vs. normal kidneys.

expression was mainly found on the peritubular capillaries. ICAM-1 expression in the glomeruli also increased strongly after 12 and 24 hours, as shown in Figure 5. ICAM-1 in cortex in rats with ARF induced by $HgCl_2$ was not different from normal kidneys.

The OSOM

At 2 and 6 hours, no differences between both groups could be observed. In the OSOM, differences in ICAM-1 expression between both models were observed at 12 hours (number of fields of ICAM-1 at hour 12,

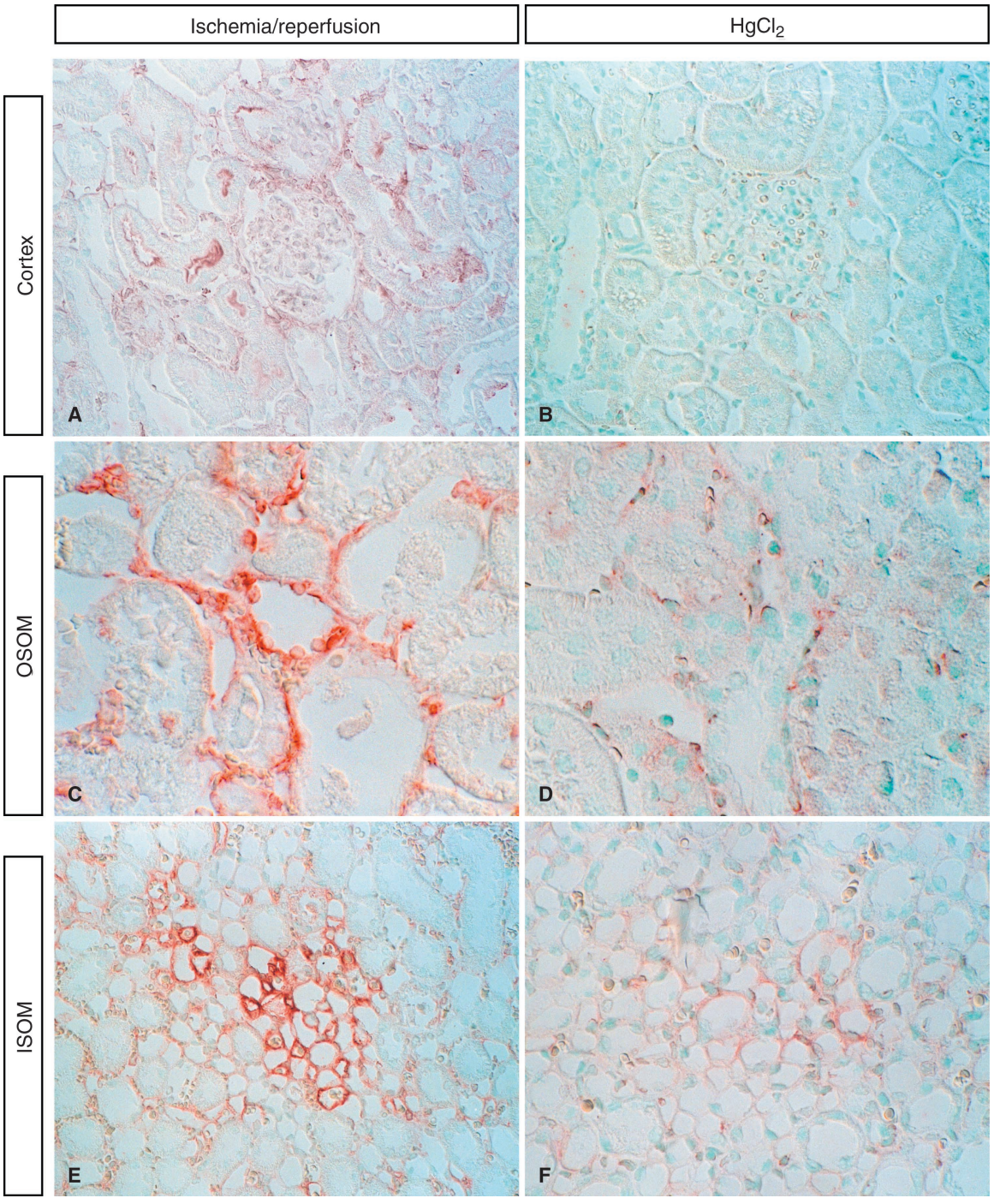

Fig. 5. Expression of intercellular adhesion molecule-1 (ICAM-1) in the different compartments of the kidney after ischemic (left) and $HgCl_2$ (right) acute renal injury. In the cortex (*A* and *B*), ICAM-1 expression is increased 24 hours after ischemic injury. ICAM-1 expression after $HgCl_2$-induced acute renal failure (ARF) is not increased. In the outer stripe of the outer medulla (OSOM) (*C* and *D*), no differences between both groups were observed except for a difference at 12 hours after reperfusion. In the inner stripe of the outer medulla (ISOM) (*E* and *F*), ICAM-1 is up-regulated soon after ischemia/reperfusion (I/R) injury. No increase in ICAM-1 expression could be found after $HgCl_2$-induced ARF at 2, 6, 12, and 24 hours.

$HgCl_2$ = 100 ± 5 vs. I/R injury = 230 ± 5; $P < 0.0001$) (area% of ICAM-1 at hour 12, $HgCl_2$ = 0.6 ± 0.1%; I/R injury = 2.3 ± 0.2%; $P < 0.005$). ICAM-1 in OSOM in rats with ARF induced by $HgCl_2$ was not different from untreated animals in the first 12 hours after injury. At 24 hours, proteinaceous materials in the tubular lumen stained weakly.

The ISOM

In ISOM, ICAM-1 expression was significantly increased at all investigated time points in the I/R group

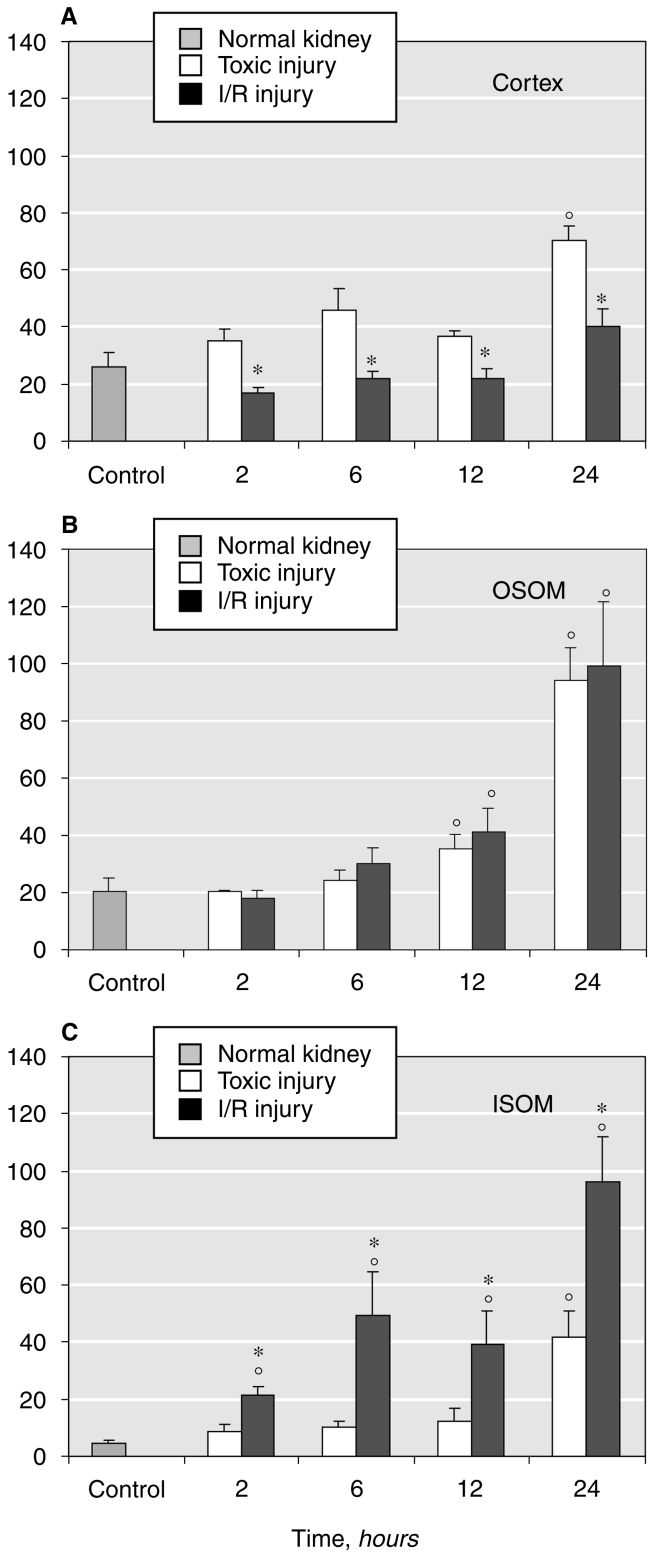

Fig. 6. Monocytes and macrophages after acute renal injury. Monocytes and macrophages were stained with the monoclonal antibody ED-1. In the cortex (*A*), more monocytes and macrophages were found after $HgCl_2$-induced acute renal failure (ARF). In the outer stripes of the outer medulla (OSOM) (*B*), no differences between both groups were observed. In the inner stripe of the outer medulla (ISOM) (*C*), soon after injury, more monocytes and macrophages were found after ischemia/reperfusion (I/R) injury. *$P < 0.05$, I/R injury kidneys vs. $HgCl_2$; °$P < 0.05$, injured kidneys vs. normal kidneys.

compared to $HgCl_2$. After 2 hours, we found a significant rise in ICAM-1 expression in the ISOM, mainly on the endothelial cells of the vasa recta (number of fields of ICAM-1 at hour 2, $HgCl_2 = 2 \pm 2$; I/R injury $= 85 \pm 9$; $P < 0.01$; at hour 6, $HgCl_2 = 6 \pm 3$; I/R injury $= 208 \pm 22$; $P < 0.0001$; at hour 12, $HgCl_2 = 45 \pm 12$; I/R injury $= 287 \pm 25$; $P < 0.0001$; at hour 24, $HgCl_2 = 27 \pm 5$; I/R injury $= 337 \pm 33$; $P < 0.005$). The increase in ICAM-1 expression in the ISOM after I/R injury was also reflected in the positive area (area% of ICAM-1 at hour 2, $HgCl_2 = 0.0001 \pm 0.0002\%$; I/R injury $= 0.76 \pm 0.2$; $P < 0.05$; at hour 6, $HgCl_2 = 0.02 \pm 0.01\%$; I/R injury $= 3.2 \pm 0.9$; $P < 0.05$; at hour 24, $HgCl_2 = 0.06 \pm 0.05\%$; I/R injury $= 7.7 \pm 2$; $P < 0.05$).

The ICAM-1 expression in ISOM, in rats with ARF induced by $HgCl_2$, was not different from normal kidneys following the first 24 hours after injury.

Leukocytes in the injured kidney

PMN cells. In all compartments of the kidney, only scarce neutrophils (hematoxylin and eosin staining) could be noticed at the early phase up to 24 hours after ischemia/reperfusion injury as well as in the early phase after a toxic insult induced by $HgCl_2$, and were not significant different from normal kidneys at any investigated time point.

The cortex

Monocytes/macrophages. Kidneys subjected to $HgCl_2$ had more monocytes/macrophages (Fig. 6) in the cortex compared to kidneys after I/R injury at all time points. This difference between both groups was observed very early after injury (2 hours after injury, I/R injury $= 17 \pm 3$ cells/mm^2 vs. $HgCl_2 = 35 \pm 4$ cells/mm^2; $P < 0.05$; 6 hours after injury, I/R injury $= 21 \pm 6$ cells/mm^2 vs. $HgCl_2 = 46 \pm 15$ cells/mm^2; $P < 0.05$; and 12 hours after injury, I/R injury $= 21 \pm 6$ cells/mm^2 vs. $HgCl_2 = 36 \pm 4$ cells/mm^2; $P < 0.05$). In the first 12 hours, there was no significant difference in monocytes/macrophages between the $HgCl_2$ and the normal kidneys. In addition, there was no significant difference in monocytes/macrophages accumulation between the I/R injury group and the normal kidneys. Twenty-four hours after the toxic insult, the accumulation of monocytes/macrophages became significant compared to normal kidneys and compared to ischemic kidneys (I/R injury $= 40 \pm 6$ cells/mm^2 vs. $HgCl_2$ 70 ± 5 cells/mm^2; $P < 0.05$).

The OSOM

In the OSOM, a difference between both models of ARF was not found. In the I/R injury group, more mono-

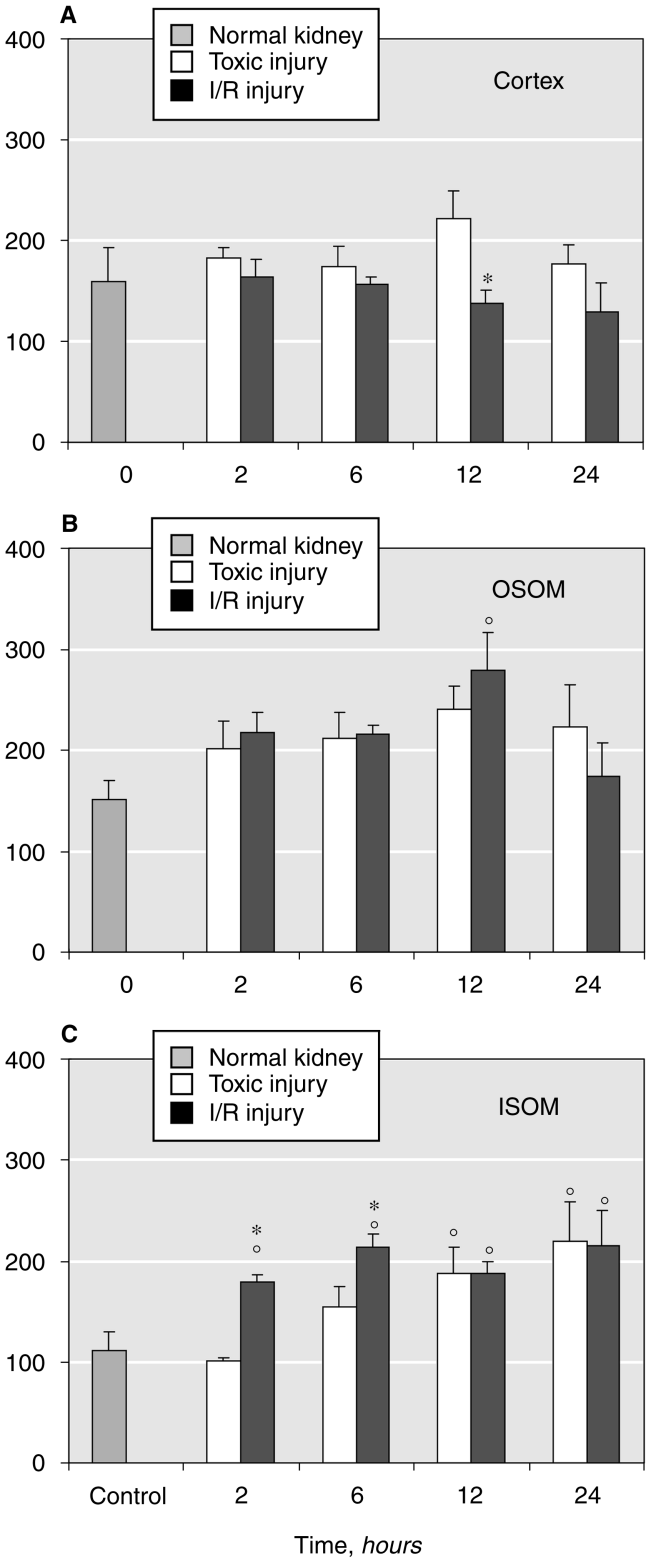

Fig. 7. T cells after acute renal injury. T cells were stained with the monoclonal antibody HIS-17. In the cortex (*A*), no accumulation of T lymphocytes was observed in the kidneys with acute renal failure (ARF), compared to noninjured kidneys. In the outer stripe of the outer medulla (OSOM) (*B*), no differences between both groups were observed. In the inner stripe of the outer medulla (ISOM) (*C*), soon after injury, more T-cell accumulation was found after ischemia/reperfusion (I/R) injury. *$P < 0.05$, I/R injury kidneys vs. $HgCl_2$; °$P < 0.05$, injured kidneys vs. normal kidneys.

cytes/macrophages were found, compared to normal kidneys, at 12 hours after reperfusion (12 hours after injury, I/R injury = 41 ± 8 cells/mm^2 vs. normal kidneys, 20 ± 5 cells/mm^2; $P < 0.05$). In the $HgCl_2$ group, more monocytes/macrophages were found, compared to normal kidneys (from hour 12, $HgCl_2$ = 35 ± 5 cells/mm^2 vs. normal kidneys = 20 ± 5 cells/mm^2; $P < 0.05$). At hour 12 and 24, no differences between both ARF models were observed.

The ISOM

In the ISOM, a significant increase of monocytes/macrophages could be observed very early after the onset of the I/R. A significant difference between the I/R injury group and normal kidneys was observed early after injury (2 hours after injury, I/R injury = 21 ± 3 cells/mm^2 vs. normal kidneys, 4 ± 1 cells/mm^2; $P < 0.05$). In contrast, within the first 12 hours after $HgCl_2$ exposure, these injured kidneys had no increase in the number of monocytes/macrophages in the ISOM, compared to control kidneys. Already early after injury, a significant difference between both ARF groups could observed (2 hours after injury, I/R injury = 21 ± 3 cells/mm^2 vs. $HgCl_2$, 8 ± 2 cells/mm^2, $P < 0.05$).

T cells. In the cortex and in the OSOM, no differences in T-lymphocyte accumulation between IRI, $HgCl_2$ and normal kidneys were observed during the first 24 hours after injury (Fig. 7).

In the ISOM, a significant increase of T lymphocytes could be observed very early after the onset of the I/R compared to normal kidneys (at hour 2, I/R injury = 179 ± 8 cells/mm^2 vs. normal kidneys = 111 ± 20 cells/mm^2; $P = 0.01$). A significant difference in T-lymphocyte accumulation could be found between both models of ARF (at hour 2, I/R injury = 179 ± 8 cells/mm^2 vs. $HgCl_2$, 100 ± 3 cells/mm^2; $P < 0.05$).

Cytotoxic T cells. No early accumulation/infiltration of CD8+ cells after ARF could be observed in the different compartments of the kidney. The number of cells in the injured kidneys was not different from control animals at any study time point.

DISCUSSION

The course of ARF, induced by I/S or by toxic injury ($HgCl_2$), is characterized by acute tubular necrosis of the S_3 segment with a transient rise in serum creatinine, commensurable in both models [1, 2, 14, 17, 18]. Similarly, the pattern and the composition of the interstitial leukocyte infiltrate after both types of renal insults are comparable in the first 10 days after the onset of injury.

Indeed, in the zone of damage, namely the OSOM, this infiltrate starts 12 to 24 hours following the initial insult and becomes most prominent after 10 days [1, 2]. Macrophages and T lymphocytes preferentially infiltrate in this most injured zone of the kidney in both models. Notwithstanding such striking resemblance in the course of injury and infiltration, there is a discrepancy between the efficacy of antiadhesion therapies (anti-ICAM-1) in renal I/S injury and $HgCl_2$-mediated toxic ARF models [7, 12]. Indeed, we found an early up-regulation of the adhesion molecule ICAM-1 particularly in the ISOM after I/R injury, going along with an accumulation of monocytes/macrophages compared to T cells in this zone of the kidney. This observation contrasts sharply with $HgCl_2$-induced ARF, in which almost no ICAM-1 expression and early leukocyte accumulation in the ISOM could be observed. Hence, the effect of anti-ICAM-1 should not be sought in the alterations in the cortex nor in the OSOM, but in the region of the ISOM, at the level of the microcirculation. These results provide a scientific base for the proposition of Kelly et al [7] who speculated that indeed ischemia could enhance ICAM-1 expression in the vasa recta, which increases adhesion of leukocytes to these vessels resulting in their plugging by red and white blood cells and the sustained medullary ischemia. In addition Wolgast et al [19] already suggested that I/R injury causes alterations in the structure and function of the vascular membranes, leading to adherence of leukocytes and red blood cells in these medullary vessels [19]. Activated leukocytes are less deformable, which may cause capillary obstruction and prevent full restoration of blood flow after reperfusion, resulting in prolonged tissue ischemia [20–24]. Activation status of the inflammatory cells (T cells and macrophages) will contribute to their adhesion. These cells become less deformable and a source of local inflammatory reaction.

As early as 1850, Reinhard [25] noted that the kidneys of patients dying from "the acute Bright's disease" had a hyperemic medulla with a pale cortex. Therefore, in the past, many authors have focused attention on the renal cortex in attempts to explain the functional deficit in ischemic ARF [26]. The inability to produce hyperosmolar urine and to secrete potassium, both common features of ischemic ARF, however, suggests an impairment of the function of the renal medulla [27–31]. Measurement of the renal blood flow with ^{86}Rb (rubidium) extraction method showed that the postischemic cortical blood flow is well preserved, whereas the postischemic medullary blood flow, 10 minutes after reperfusion, is approximately 10% of the nonischemic controls [19]. More than 100 years later after the observation of Reinhard [25], the concept of a decreased medullary blood flow as a consequence of medullary congestion has been demonstrated by many investigators. Congestion of the outer medulla has been previously described in conjunction with tubular injury induced by ischemia [27–30, 32–34] and has been documented following the infusion of indomethacin and radiocontrast [35], as well as after ARF induced by cisplatin [36, 37]. In contrast, after a toxic insult induced by $HgCl_2$, the medullary flow is preserved, or even increased [14, 38, 39]. $HgCl_2$ becomes directly tubulotoxic, partially due to exposure of tubular epithelial cells to high concentration of the compound at the luminal side of the tubule [40, 41]. Conger and Falk [39] demonstrated that single-nephron glomerular filtration rate (GFR) after $HgCl_2$ injection was unchanged when measured at Bowman's space, but, when measured in the late proximal tubule, single-nephron GFR was significantly reduced. The authors concluded backleak of tubular fluid, secondary to the necrotic lesions to be the primary cause of decreased GFR and rise in serum creatinine in $HgCl_2$ ARF, while alterations of renal hemodynamics seemed rather unimportant in this model, with even increased medullary flow [38]. Our observation of ICAM-1 up-regulation, especially in the ISOM with an increased number of accumulated monocytes/macrophages and T cells, is concomitant with the long-lasting observation of the decreased medullary blood flow after ischemic renal injury [42]. In contrast, we found neither ICAM-1 up-regulation nor early leukocyte accumulation after $HgCl_2$ in this zone, concomitant with the observation of the Conger and Falk, demonstrating a well-preserved medullary blood flow after $HgCl_2$. All these observations support the lack of effect of antiadhesion approach (anti-ICAM-1/anti-LFA-1) in this toxic model [12].

As ICAM-1 is distributed widely on endothelial cells, on different leukocyte subsets, and even on the brush borders of the proximal tubular cells in the normal and the ischemic kidney [43], the results of the experiments using anti-adhesion approach have to be considered cautiously. A working mechanism of antiadhesion treatment may consist in the prevention of tubular obstruction caused by tubular cast formation as demonstrated by Goligorsky and DiBona [44] by the use of arginine glycine aspartic acid (RGD) peptides. However, this mechanism seems less obvious in the very early phase of I/R injury. First, tubular cell necrosis is found in both toxic and I/R models, in a later phase after the onset of injury [45]. In both models, tubular necrosis/casts appeared between 12 hours and 24 hours after the onset of injury, at a time when the anti-ICAM-1 treatment had already provided protection in I/R injury. Second, tubular necrosis/obstruction is present in the $HgCl_2$ model, in which an anti-adhesion approach is not protective.

CONCLUSION

We demonstrated early ICAM-1 up-regulation after I/R injury in the ISOM. In contrast, no early changes in ICAM-1 expression after $HgCl_2$ were observed. In

addition, we found an increase in monocyte/macrophage and T-cell accumulation in the ISOM early after I/S injury, whereas such monocytes/macrophages and T-cell accumulation after $HgCl_2$ in the ISOM could not be found. These data suggest that the ISOM is a critical zone of the kidney in pathophysiology of I/R injury [42]. These observations bring new insight in the mechanisms why anti-adhesion molecules in I/R injury of the kidney were successfully and may explain the failure of the same combined treatment with monoclonal antibodies LFA-1/ICAM-1 in a toxic model of $HgCl_2$-induced ARF.

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